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Control of Immunodeficiency and Lymphoproliferation in Mouse AIDS: Studies of Mice Deficient in CD8$^+$ T Cells or Perforin

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CD8$^+$ T cells were previously shown to be important in preventing lymphoproliferation and immunodeficiency following infection of murine AIDS (MAIDS)-resistant mice with the LP-BM5 mixture of murine leukemia viruses. To further evaluate the mechanisms contributing to MAIDS resistance, we studied mice lacking CD8$^+$ T cells or deficient in perforin due to knockout of the β2-microglobulin (β2M) or perforin gene, respectively. In contrast to wild-type, MAIDS-resistant controls, B10.A mice homozygous for the β2M mutation and B10.D2 mice homozygous for the perforin mutation were diagnosed as having MAIDS by 5 to 8 weeks after infection by the criteria of lymphoproliferation, impaired proliferative responses to mitogens, and changes in cell populations as judged by histopathology and flow cytometry. Unexpectedly, there was no progression of lymphoproliferation through 24 weeks, even though immune functions were severely compromised. Expression of the defective virus responsible for MAIDS was enhanced in spleens of the knockouts in comparison with wild-type mice. These results demonstrate that perforin-dependent functions of CD8$^+$ T cells contribute to MAIDS resistance but that other, non-CD8-dependent mechanisms are of equal or greater importance.

The consequences of challenging adult mice of different inbred strains with a retrovirus mixture that induces mouse AIDS (MAIDS) are highly variable, but the strain responses can be grouped into three general categories: sensitive, intermediate, and resistant (9, 10, 13, 21, 24). Sensitive strains, such as C57BL/6 (B6) and C57L, exhibit substantial lymphoproliferation and immunodeficiency within 4 weeks of infection, whereas resistant strains, such as A/J and RIIIS/J, develop no or very late onset disease (19–21). Intermediate responses, like those of (B6 × CBA/N)F_1 and BALB/c, include disease with prolonged latency, slow progression, or variable individual mouse sensitivity (10, 13, 21).

Strain differences in response to this infection are genetically determined, with genes of the major histocompatibility complex (MHC) having, in many circumstances, the major influence on susceptibility to MAIDS. Within the MHC, class I genes at the *D* end are most clearly associated with resistance to MAIDS (21, 22), but modulating effects of class II genes, both *I-A* and *I-E*, are well documented (23). Genes outside of the MHC can also critically affect the development of disease (20, 21). The mechanisms by which either MHC or non-MHC genes control disease are not well understood, although the ability to control spread and expression of the etiologic replication-defective virus, designated BM5def (3, 4) or Du5H (1), appears to be crucial (13, 19, 26). In some cases using mixed virus stocks for challenge, control of BM5def—and thus of disease—may be linked to restriction of helper virus replication (19, 20).

Previous studies showed that CD8$^+$ cells were important for control of BM5def and development of MAIDS. Normal A/J mice eliminated the defective virus and showed no signs of disease, while mice depleted of CD8$^+$ cells by chronic treatment with anti-CD8 monoclonal antibody (MAb) had modest splenomegaly due to MAIDS, and the BM5def genome was readily detectable (19). The mechanisms responsible for this effect of CD8$^+$ T cells could include either perforin- or Fas-dependent cytotoxicity for BM5def-infected cells or the production of cytokines that restrict virus replication and spread (reviewed in reference 15). Studies indicating that CD8$^+$ cytotoxic T lymphocytes (CTL) could be raised against the defective virus Gag protein (28) suggested direct lysis of infected targets as the most likely mechanism of protection. In the present study, we reassessed the role of CD8$^+$ T cells in resistance to MAIDS by using β2-microglobulin (β2M) knockouts and examined the possible contributions of perforin-dependent cytotoxicity in perforin-deficient mice.

MATERIALS AND METHODS

Mice. B6 and B10.D2/SnJ (B10.D2) mice were purchased from the Jackson Laboratory (Bar Harbor, Maine). B10.A/SgSnAi mice were from the colony of R. Schwartz (National Institute of Allergy and Infectious Diseases). Mice bearing a germ line disruption of the β2M gene (30) were obtained at the third backcross from 129 to C57BL/6 from Maarten Zijlstra and Rudolf Jaenisch (Whitehead Institute, Cambridge, Mass.). The mice were backcrossed to B6 mice for another five generations (N9) before intercrossing to generate homozygous knockouts (−/−) and controls (+/− and +/+). N9 heterozygotes were backcrossed to B10.A/SgSnAi for five generations before intercrossing to generate B10.A knockouts. Typing of mice for the knockout was performed by Southern blotting of DNA prepared from tail biopsies, using probe and conditions described previously (30).

B6 mice homozygous for a disruption of the perforin gene were generated by using B6 ES cells as previously described (14). Knockouts bearing the *H-2D^d* haplotype were generated by crossing deficient males with normal B10.D2 females, generating F_2 mice, and selecting mice homozygous for *H-2D^d* and for the knockout. Subsequent breeding and testing of the B10.D2 knockouts were performed at the University of Geneva.

Viruses and virus assays. Stocks of LP-BM5 murine leukemia viruses (MuLVs) that contain a mixture of nonpathogenic ecotropic and mink cell focus-inducing (MCF) viruses and a etiologic replication-defective virus (BM5def) were prepared from the G6 clone of SC-1 cells as described previously (4). Mice were inoculated intraperitoneally with 0.1 ml of virus stock at 4 to 6 weeks of age. In studies of β2M knockout mice, infectious center assays for ecotropic and MCF viruses were performed with mitomycin-treated suspensions of spleen cells as detailed elsewhere (10).

In studies of the perforin knockout mice, frequencies of virus-producing cells

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TABLE 1. Development of MAIDS in B10.A mice[a]

Wk after infection	β2M genotype	Spleen wt (mg)	Stage of disease		Ecotropic virus recovery (PFU $[\log_{10}]/10^7$ cells)	Splenic $CD8^+$ cells (%)
			FACS	Path		
0	+/+	90, 120	N	N	<1.0, <1.0	13, 14
	+/−	100, 110	N	N	<1.0, <1.0	12, 15
	−/−	100, 120	N	N	<1.0, <1.0	<1.0, <1.0
5	+/+	80, 90, 100	N, R	R	ND	12, 13, 15
	−/−	90, 230, 240, 270	R, 1	1	ND	<1.0, <1.0, <1.0, <1.0
8	+/+	90, 90	N	ND	<1.0, <1.0	11, 11
	+/−	90, 90	N	ND	<1.0, <1.0	10, 13
	−/−	180, 220, 250, 320	1	1	5.1, 5.5, 5.5, 5.7	<1.0, <1.0, <1.0, <1.0
13	+/+	70, 70	N	R	<1.0, <1.0	10, 14
	+/−	250, 280	1	1	4.8, 4.5	2, 5
	−/−	200, 310	1	R, 1	4.6, 4.4	<1.0, <1.0
24	+/+	90	ND	N	ND	ND
	+/−	370	ND	1	ND	ND
	−/−	230, 270, 300	ND	1	ND	ND

[a] B10.A mice of the indicated β2M genotypes were infected with LP-BM5 MuLV and killed for studies of lymphoproliferation, stage of disease, and frequency of spleen cells producing infectious ecotropic virus. The criteria used to stage disease by FACS or histopathology (Path) have been detailed previously (see Materials and Methods). Values indicate individual or average values for two to four mice per point. ND, not done.

were determined by immunochemical assays (13a). Briefly, mitomycin-treated single-cell suspensions prepared from spleen were added to subconfluent SC-1 cells in the presence of Polybrene (8 μg/ml). Nonadherent cells were washed off 1 day later, and culture of the monolayer was continued for another 4 days. The cells were then fixed, and foci of infection were visualized by incubation with hybridoma supernatants containing MAb to MuLV determinants followed by horseradish peroxidase-labeled rabbit anti-mouse immunoglobulin (Ig) (Southern Biotechnology Associates, Birmingham, Ala.). The anti-MuLV antibodies, a gift from B. Chesebro (5), included MAb 548, which is broadly cross-reactive with Gag p12 proteins of most MuLVs tested but not with BM5def p12. For the purposes of this study, 548 positivity is equated with MuLV infection.

Transcripts for BM5def in spleen cells were detected by reverse transcriptase PCR (RT-PCR) techniques, using primers, probe, and conditions as described elsewhere (6–8).

Studies of infected mice. At autopsy, mice were bled, spleen and cervical lymph node weights were measured, and selected tissues were formalin fixed for histopathologic studies or frozen at −70°C for later preparation of DNA. Single-cell suspensions prepared from spleen were used for infectious center tests for MuLV and were stained for fluorescence-activated cell sorting (FACS), using a panel of antibodies useful in staging the progression of MAIDS (10, 17), including antibodies to Thy-1, Ig kappa light chain, CD11b (Mac-1), IgG, CD4, CD45R (B220), and the IgG Fc receptor. Additional antibodies were used to verify the genotyping for β2M (anti-*H-2D*^{d}) and to examine T-cell subsets. FACS and histopathologic criteria used to stage the progression of MAIDS have been described elsewhere (10, 17). Briefly, N indicates normal; R indicates changes consistent with a reaction to infection but with insufficient changes to be diagnosed as MAIDS; and stages 1, 2, and 3 indicate changes consistent with a diagnosis of MAIDS of increasing severity. Reactive changes include some enlargement of the periarteriolar lymphoid sheath (PALS) and moderate enlargement of follicles. Stage 1 disease is associated with marked enlargement and distortion of the PALS, often to an hourglass shape, and compression of the surrounding red pulp. The population of small lymphocytes normally found in the PALS is almost completely replaced with cells of follicular origin. Stages 2 and 3 of disease are marked by even greater enlargement of the PALS and almost complete loss of the red pulp. Stage 3 disease is associated with prominent extralymphoid infiltrates in the lung, liver, or kidneys as well as increased levels of immunoblasts, plasmacytoid cells, and plasma cells in spleen and lymph node.

Suspensions of spleen cells stimulated with concanavalin A (ConA) or lipopolysaccharide, or stimulated through the T-cell receptor (TCR) with anti-TCRα/β MAb H57-597, were tested for proliferative responses as described previously (6).

RESULTS

Studies of B10.A-β2M knockouts. The contribution of $CD8^+$ T cells to MAIDS resistance of A/J mice was previously assessed in mice depleted of this T-cell subset by chronic administration of anti-CD8 MAb (19). While these studies showed that depleted mice were susceptible to MAIDS, the interpretation was somewhat complicated by the need to treat mice with anti-CD4 MAb during the initiation of the protocol to prevent immune responses to the xenogenic antibody. In addition, with this regimen it is difficult to maintain long-term depletion of $CD8^+$ cells in mice that have not been thymectomized.

The development of mice deficient in $CD8^+$ T cells due to disruption of the β2M gene provided a new opportunity to examine the full range of functional attributes of $CD8^+$ cells in relation to control of MAIDS-associated lymphoproliferation and immunodeficiency. Since the initial studies of the effects of $CD8^+$ cells in MAIDS were performed with the resistant strain A/J (19), we examined the effects of the β2M knockout in the *H-2*-identical, MAIDS-resistant strain, B10.A. Previous studies showed these mice to develop MAIDS with a low frequency and a prolonged time course (21).

As expected, most wild-type B10.A mice showed no signs of MAIDS through 24 weeks after infection as determined by FACS, histopathology, and functional criteria (Table 1). In contrast, mice homozygous for the β2M knockout exhibited clear signs of MAIDS by both criteria within 5 weeks of infection. Similar results were obtained in comparisons of +/+ and −/− mice tested at 6 (not shown) and 12 weeks after infection (Table 2). Although no effect of heterozygosity for the knockout was seen at 8 weeks after infection, in later assays the spleen weights and staging of disease were similar for heterozygotes and homozygous knockouts; however, progression of lymphoproliferation was minimal such that spleen weights of +/− and −/− mice were less than 500 mg through 24 weeks after infection. The observations that heterozygosity at β2M was associated with defects comparable with those seen in $\beta2M^{-/-}$ mice was unforeseen but is probably related to the effects of heterozygosity on quantitative expression of MHC class I molecules. Previous studies clearly demonstrated that MHC class I expression is reduced in mice heterozygous for the gene disruption (11, 18). This is due to the fact that β2M is required for normal intracellular processing of class I molecules and that the supply is limited when only one allele of β2M can be expressed. The deficit in MHC class I expression exhibited by $\beta2M^{+/-}$ mice is not associated with a deficit in peripheral $CD8^+$ T cells, however, as comparable frequencies are present in the spleens and lymph nodes of +/− and +/+ mice (18) (Table 1).

FACS analysis of spleens from infected −/− mice, using antibodies to CD8 and to *H-2D*^{d}, demonstrated that the infection did not result in development and peripheral seeding of

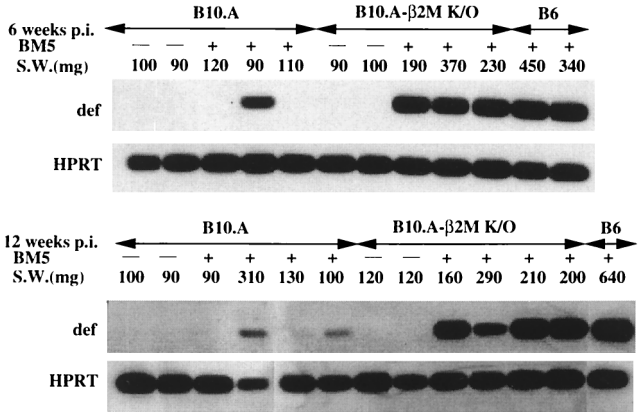

FIG. 1. RT-PCR analyses of BM5def expression in spleen cells of B10.A wild-type and β2M knockout (K/O) mice and B6 mice. Mice infected for the indicated time periods and uninfected age-matched controls were examined for spleen weights (S.W.), and samples prepared from spleen were evaluated for transcripts of BM5def (def) and hypoxanthine phosphoribosyltransferase (HPRT), used as an internal control. On original films, BM5def transcripts were detected in samples from spleens of infected B10. A mice with spleen weights of 310, 130, and 100 mg. p.i., postinfection.

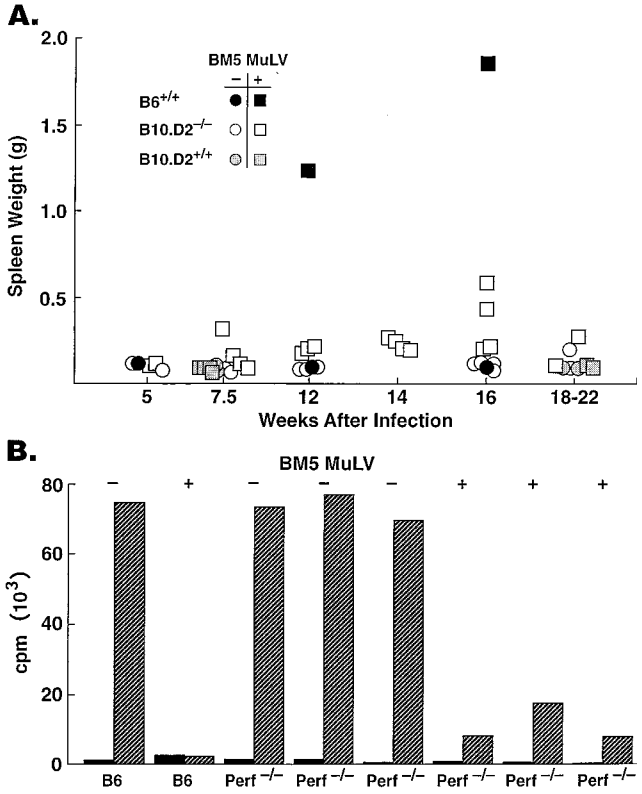

FIG. 2. Development of lymphoproliferation and immunodeficiency in perforin knockout (Perf$^{-/-}$) mice. (A) Mice of the indicated strains and genotypes were infected with LP-BM5 MuLV (+) and examined for spleen weight along with age-matched uninfected controls (−) at the indicated times after infection. Points indicate values for individual mice. (B) Spleens from mice examined at the 12-week time point in panel A were tested for in vitro proliferative responses to ConA (hatched bars) or to medium alone (solid bars).

CD8$^+$ cells (Table 1) or in induction of MHC class I expression (data not shown).

Assays of spleen cells for infectious ecotropic virus showed that the frequency of virus-producing cells was low for +/+ mice at both times tested but that at 13 weeks, +/− and −/− mice expressed equal levels of virus (Table 1).

RT-PCR analyses of BM5def transcripts in spleens of mice from Table 1 infected for 13 weeks showed that expression was below the limits of detection in +/+ mice but that transcripts were readily detectable in spleens of both +/− and −/− mice (not shown). In a second experiment, RT-PCR analyses revealed substantial levels of BM5def transcripts in the spleens of one of two wild-type mice and all of three −/− mice infected for 6 weeks (Fig. 1). At 12 weeks after infection, variably low levels of BM5def transcripts were detected in spleens of three of four wild-type mice and at higher levels in spleens of all four −/− mice. This result indicates that +/+ mice were able to clear or limit the spread of BM5def but that expression was usually substantially higher in the knockouts and correlated with increased spleen weights.

TABLE 2. Proliferative responses of spleen cells to mitogenic stimulation and TCR cross-linking[a]

Mouse	LP-BM5 infected	Spleen wt (mg)	Proliferation (cpm, 10^{-3})			
			ConA	Lipopoly-saccharide	TCRα/β	PMA + TCRα/β
B10.A	−	100	370	215	225	370
		90	375	195	183	393
	+	90	418	214	234	373
		310	56	126	50	178
		130	461	236	242	307
		100	445	194	214	380
B10.A-β2M$^{-/-}$	−	120	314	210	232	251
		120	207	132	161	236
	+	160	80	21	81	148
		290	29	2	51	112
		210	19	64	24	59
		200	12	49	17	60
B6	+	1,170	5	6	5	18
		640	8	6	4	17

[a] Spleen cells from uninfected mice or mice infected for 12 weeks were stimulated in vitro with mitogen, anti-TCRα/β MAb, or anti-TCR MAb and phorbol myristate acetate (PMA). Cells were harvested at 72 h. Numbers indicate mean values for triplicate cultures.

Functional analyses showed that proliferative responses of spleen cells to T- and B-cell mitogens and to cross-linking of the TCR were unaffected in all wild-type B10.A mice infected with LP-BM5 viruses for 6 weeks (data not shown) and most infected for 12 weeks (Table 2). The single wild-type-infected mouse with a 310-mg spleen had impaired proliferative responses in keeping with stage 1 level of disease progression. In contrast, proliferative responses were substantially decreased in all CD8-deficient mice infected for 6 weeks (not shown) or 12 weeks (Table 2). It should be noted that the impairment exhibited by the knockouts was less severe than that shown by B6 mice infected for the same period of time. The impaired proliferation by spleen cells of mice with MAIDS in response to TCR cross-linking was partially restored by costimulation with phorbol myristate acetate, suggesting that the T-cell defect in infected mice reflected, at least in part, impaired activation of phospholipase Cγ1.

The results obtained with wild-type mice and homozygous mutants suggest that CD8$^+$ T cells are critically important for resistance to MAIDS. The level at which this effect may be mediated, however, is not clear. Since the frequencies of CD8$^+$ cells are not detectably different in heterozygous mutant and normal mice and heterozygotes develop disease just as severe as that observed in β2M$^{-/-}$ mice, the defect must lie at some

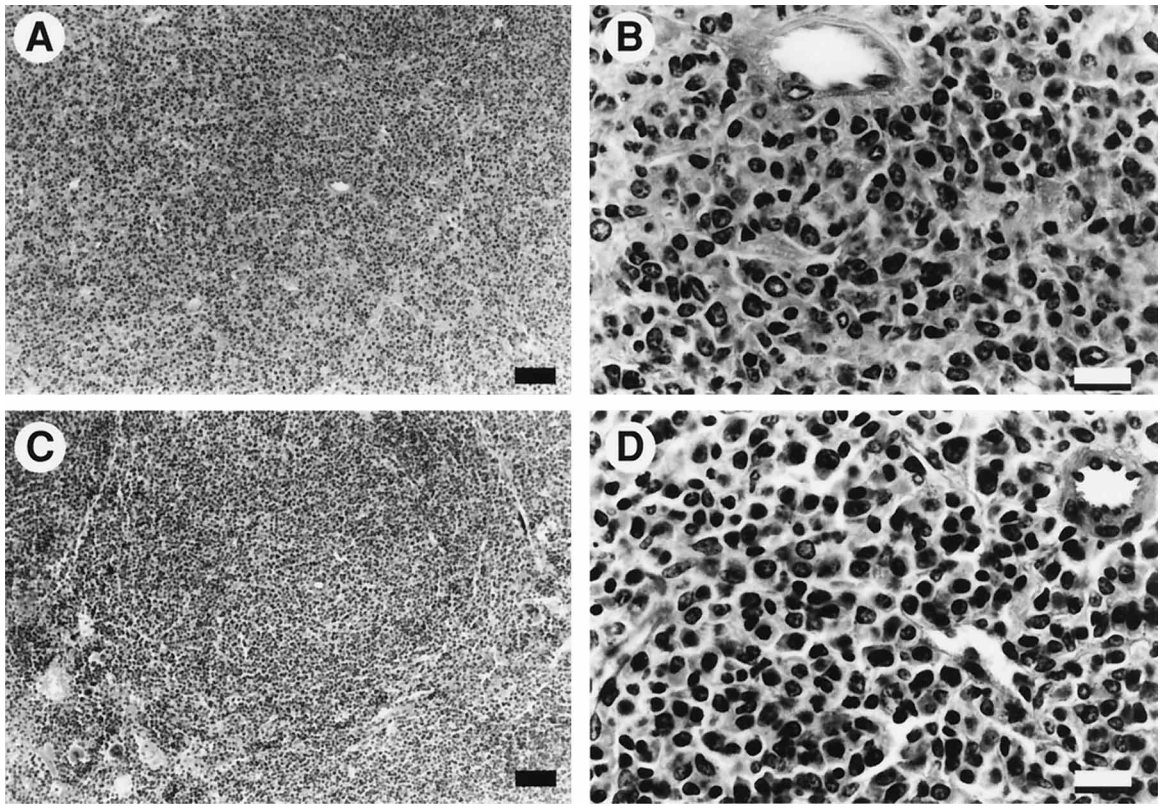

FIG. 3. Histologic comparisons of spleens from B10.D2 wild-type (A and B) and perforin$^{-/-}$ (C and D) mice infected for 12 weeks and weighing 1,240 and 220 mg, respectively. (A) An enlarged splenic follicle covers the entire field. The central arteriole is surrounded by a population of cells typical of advanced MAIDS. Hematoxylin and eosin stain; bar, 0.3 μm. (B) The central arteriole at the top is surrounded by immunoblasts, plasmacytoid cells, and occasional plasma cells. Hematoxylin and eosin stain; bar, 30 μm. (C) The enlarged splenic follicle is surrounded by red pulp; note megakaryocytes in the lower left and at the right. The central arteriole is in the middle and surrounded by lymphoid cells typical of MAIDS. Hematoxylin and eosin stain; bar, 0.3 μm. (D) The cells surrounding the central arteriole at the upper right corner are similar to those shown in panel B. Hematoxylin and eosin stain; bar, 30 μm.

level other than the frequency of CD8$^+$ T cells. The density of MHC class I molecules is reduced on cells of heterozygous knockouts (reference 11 and 18 and data not shown). It is thus possible that antigen-presenting cells or infected targets expressing reduced levels of MHC class I molecules complexed with antigenic peptides may be less effective at sensitizing CD8$^+$ cells, that infected cells may prove to be inferior targets for sensitized CD8$^+$ cells, or some combination of these possibilities. It is also possible that reduced expression of MHC class I molecules in the thymus is responsible for impaired positive selection of T cells (2, 16) required for a protective response to BM5def.

In both +/− and −/− mice, susceptibility was associated with enhanced expression of ecotropic and defective viruses, impaired immune function, and early lymphoproliferation; however, the spleen weights of +/− and −/− mice at late times after infection were similar to those observed at early time points and were much less than those of MAIDS-susceptible B6 mice.

Studies of B10.D2 perforin-deficient mice. Mechanisms by which CD8$^+$ T cells confer resistance to viruses have been shown to include antigen-specific, perforin-dependent cytotoxic activity against infected targets (14). To evaluate the possible contribution of this mechanism to resistance to MAIDS, we tested B10.D2 mice that carry a knockout of the perforin gene but which have normal numbers of CD8$^+$ T cells (14). Normal B10.D2 mice are highly resistant to MAIDS, with characteristic disease first being seen at 39 weeks after adult infection (data not shown). Studies of B10.D2 mice deficient in perforin showed them to develop remarkably little lymphoproliferation but significant immunodeficiency in response to infection. As shown in Fig. 2A, the spleen weights of perforin-deficient mice were slightly greater than those for controls beginning at 7.5 weeks after infection, but except for two 16-week-old mice, lymphoproliferation did not progress through 24 weeks. Even at 16 weeks, the spleen weight of infected B6 mice was more than sixfold greater than that of the perforin$^{-/-}$ animals.

To determine whether progression of immunodeficiency was comparable in infected perforin-deficient and B6 mice, spleen cells were tested for proliferative responses to stimulation with ConA (Fig. 2B) or to cross-linking of the TCR (not shown). At 12 weeks after infection, the responses of perforin knockouts were depressed but not as severely as those of infected B6 mice. Comparable levels of suppression were also seen in knockouts tested at 14 weeks after infection (data not shown). These observations indicated that functional abnormalities of infected perforin knockouts progressed together with characteristic changes detectable by histopathology or FACS in the face of limited lymphoproliferation.

Histopathologic and FACS analyses of uninfected control and knockout mice demonstrated that tissues from mice of either genotype were indistinguishable. Despite the marked differences in weight between the spleens of infected perforin-deficient and B6 mice, the histologic changes seen in spleens and lymph nodes of these two strains were unexpectedly of comparable severity (Fig. 3). Figure 3 shows that the follicular enlargement characteristic of MAIDS was much greater in the wild-type mice than in the knockout mice; the cellular composition of the follicles, however, was essentially the same. This

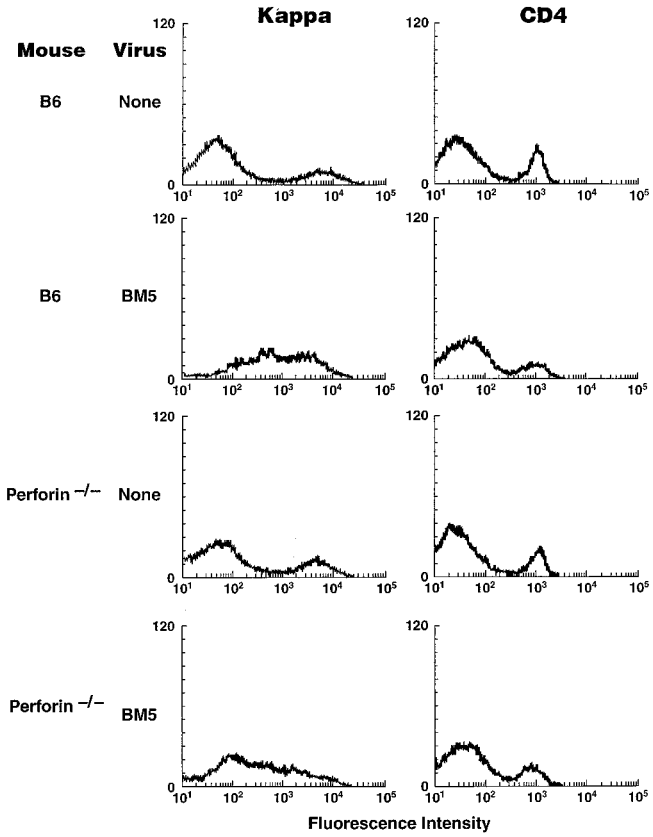

FIG. 4. FACS analyses of cells from normal or LP-BM5 MuLV-infected B6 or B10.D2 perforin knockout mice. Spleen cells were stained with antibodies to immunoglobulin kappa light chain or to CD4 and analyzed on a FACScan.

result suggested that the same factors were effecting the differentiation of B cells to immunoblasts, plasmablasts, and plasmacytoid cells but that the activated cells went through fewer rounds of proliferation in the perforin$^{-/-}$ mice. Changes seen in FACS analyses of spleens were also consistent with diagnoses of MAIDS for B6 and perforin-deficient mice. The characteristic changes included the appearance of large cells expressing Ig kappa light chain at reduced intensity and decreased frequencies of cells expressing CD4 (Fig. 4).

Virologic studies of the perforin knockouts were performed by using immunofocus assays. When assayed at 12 weeks after infection, spleens of LP-BM5 MuLV-infected perforin knockouts had frequencies of cells producing infectious ecotropic and MCF viruses comparable to those for B6 mice (not shown), indicating that the limited susceptibility to MAIDS exhibited by these mice was associated with failure to control expression of helper viruses.

mRNAs prepared from spleens of normal and infected B6 and perforin knockout mice were examined by semiquantitative RT-PCR for expression of defective virus (Fig. 5). No transcripts were detected in spleens of uninfected mice of either strain, and the levels of BM5def expression in the infected knockouts were comparable to those seen in infected B6 mice. These findings indicated that in the context of a MAIDS-resistant strain, perforin was required for control of defective as well as helper virus expression.

DISCUSSION

Previous virologic studies demonstrated that resistance to MAIDS in mice infected with the defective virus pseudotyped with competent helpers (9, 10, 13, 19, 24) or given helper free (12, 26) is associated with clearance of the defective virus (13, 19, 24, 26). In mice infected with the LP-BM5 mixed virus stocks, resistance to MAIDS also correlated strongly with lack of MCF expression and, in the most resistant mice, reduced expression of ecotropic helper virus as well (10, 20, 21). These results indicate that mechanisms that limit expression of the defective virus—by direct elimination of cells containing that virus and/or by restricting the replication of helper viruses—are likely to be of great importance to control of disease. Effects related to control of helper MuLV would clearly be irrelevant to mice challenged with defective virus only.

This study of mice deficient in CD8$^+$ T cells or perforin demonstrated that perforin-dependent functions of this T-cell subset, most likely reflecting cytotoxic activity, are of major importance to controlling expression of BM5def. The mechanisms responsible for this effect include restrictions on the spread of BM5def and, at least during the early phases of infection of β2M$^{-/-}$ mice, the ecotropic helper virus present in the LP-BM5 virus mixture.

It is noteworthy that the degree of lymphoproliferation seen in infected CD8$^+$ T-cell-deficient B10.A-β2M$^{-/-}$ mice was somewhat greater than that observed in infected A/J mice depleted of T cells by treatment with anti-CD8 MAb (19). This may be due to strain-related differences in the levels to which CD8$^+$ T cells mediate resistance to lymphoproliferation; A/J mice are unquestionably more resistant to MAIDS than are B10.A mice (21). As a second consideration, A/J mice chronically depleted of CD8$^+$ T cells were first treated short-term with anti-CD4 MAb to prevent induction of antibodies to rat Ig (19). It is possible that CD4$^+$ T cells contribute to the development of MAIDS sensitivity in A/J mice depleted of CD8$^+$ cells. Finally, CD8 is expressed on some thymic and splenic dendritic cells (29) as well as on T cells. These dendritic cells may have been eliminated by treatment with anti-CD8 MAb and may have been important as antigen-presenting cells for CD4$^+$ cells, contributing to resistance.

The observation that lymphoproliferation is strikingly modest in MAIDS-resistant strains rendered either perforin or β2M deficient indicates that other immune mechanisms, possibly involving CD4$^+$ T effectors, neutralizing antibodies, and/or cytokines, contribute substantially to virus clearance and protection from MAIDS in intact mice. Preliminary studies indicated that natural killer cell function is not augmented in β2M-deficient mice infected with LP-BM5 viruses. The conclusion that CD8$^+$ CTL may provide only one of several arms that mediate resistance to murine retrovirus-induced disorders has precedent in studies of Friend disease, a syndrome induced by a replication-defective virus distinct from BM5def and replication-competent Friend ecotropic helper viruses. Analyses of Friend virus-infected mice revealed that effective immune

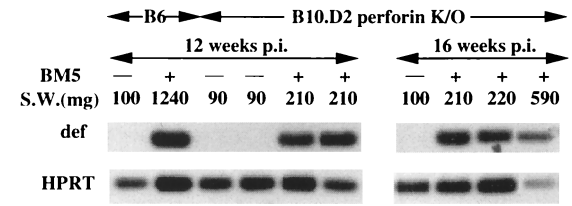

FIG. 5. RT-PCR analyses of BM5def expression in spleen cells of B10.D2 perforin knockout (K/O) and B6 mice. Spleen weights (S.W.) were determined for mice infected with LP-BM5 MuLV for the indicated times, and samples prepared from spleen were evaluated for transcripts of BM5def (def) and hypoxanthine phosphoribosyltransferase (HPRT). p.i., postinfection.

control required both humoral and cell-mediated responses (11, 27) and that infected mice failed to fully clear the infection if depleted of either $CD4^+$ or $CD8^+$ T cells (27).

It is of interest that recovery from Friend disease is associated with particular alleles at *H-2D*. Mice homozygous for *H-2D*^{b} have a high incidence of recovery from Friend virus infections, while heterozygotes for the *b* allele at *H-2D* have a low incidence of recovery. Studies of β2M-deficient mice showed no gene dosage effect on recovery from Friend disease, indicating that half levels of *H-2D*^{b} expression were sufficient for the high recovery phenotype previously associated with homozygosity at *H-2D*^{b} (11). This finding clearly differs from our analyses of B10.A mice, which showed that heterozygotes for the β2M knockout were almost as severely affected as homozygotes at 13 weeks or more after infection.

The finding that B10.A-$\beta2M^{+/-}$ mice were susceptible to MAIDS may provide an explanation for the sensitivity to disease of most F_1 mice from crosses between sensitive and resistant strains. These F_1 mice develop MAIDS but with a prolonged time course relative to the sensitive parent. Heterozygosity for an MHC class I allele associated with resistance may thus be equivalent to hemizygosity for β2M.

It has been shown that $CD8^+$ CTL directed against determinants encoded by BM5def can be elicited in MAIDS-resistant BALB/c mice (28). The importance of a virus-specific CTL response to the resistance of this strain is not known, as CTL with like specificity can be elicited in MAIDS-sensitive heterozygotes of BALB/c and MAIDS-sensitive B6 mice. Disease in the F_1 mice is delayed relative to that in B6 mice (9, 10, 21), which may reflect a contribution of CTL to control of disease.

The apparent dissociations between lymphoproliferation and immune defects in perforin$^{-/-}$ and $\beta2M^{-/-}$ mice were unexpected, although similar changes have been described for homozygous gamma interferon knockout mice infected with LP-BM5 viruses (7). In the mice described here, the immunologic abnormalities exhibited by the knockouts were relatively advanced but were not as severe as those found for normal B6 mice infected for comparable periods of time. Since lymphoproliferation was also less advanced in the knockouts, the differences in degree of progression may be more apparent than real and may simply reflect kinetic differences in the rates at which splenomegaly and immunodeficiency develop.

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